

CLAIMS

1. A method of culturing a microorganism for the synthesis of docosaehaenoic acid by the microorganism, comprising culturing a microorganism selected from the group of *Crypthecodinium cohnii* and genetically modified variants thereof with a compound selected from the group of carboxylic acids and carboxylate ions, the microorganism using the compound as a carbon source and synthesising docosaehaenoic acid.
2. A method according to claim 1, wherein said compound is acetic acid or acetate.
3. A method according to claim 1 or claim 2, wherein the compound is the main carbon source for the microorganism during the culture of the microorganism.
4. A method according to any preceding claim, wherein the microorganism is cultured in a medium, said use of the compound as a carbon source by the microorganism causing an increase in pH of the medium, and wherein the method further includes, where the compound is a carboxylic acid, addition to the medium of said carboxylic acid, or where the compound is a carboxylate ion, addition to the medium of a carboxylic acid that ionises to form said carboxylate ion, in response to the increase in pH so as to decrease the pH of the medium.

5. A method according to claim 4, wherein said addition maintains the pH substantially at a predetermined value.
6. A method according to claim 5, wherein the predetermined value is pH 6.5.
7. A method according to any one of claims 4 to 6, wherein the pH of the medium is monitored by means that produces a signal that is used to control said addition to the medium.
8. A method according to claim 7, wherein the signal is used to control addition of one or more of a nitrogen source, a phosphorus source, an amino acid, a vitamin, a salt or another growth factor during the culture of the microorganism.
9. A method according to any one of claims 4 to 7, wherein said carboxylic acid or said carboxylic acid that ionises is added to the medium in a mixture comprising a further compound.
10. A method according to claim 9, wherein the further compound is an organic acid.
11. A method according to claim 9, wherein the further compound is a lipid.

12. A method according to any one of claims 9 to 11, wherein the mixture is a waste product from an industrial process.

13. A method according to claim 9, wherein the further compound is a nitrogen source, a phosphorus source, an amino acid, a vitamin, a growth factor, a salt or a lipid.

14. A method according to any one of claims 1 to 13, wherein prior to said culture with said compound, the microorganism is grown with said compound.

15. A method according to any one of claims 1 to 14, wherein the microorganism is cultured with an organic nitrogen source, preferably with yeast extract.

16. A method according to claim 15, wherein the nitrogen source is yeast extract and the initial concentration of the yeast extract is greater than 7.5 g/l.

17. A method according to claim 16, wherein the initial concentration of yeast extract is 10 g/l.

18. A method according to any one of claims 1 to 17, wherein the microorganism is cultured with salts or osmoticants, preferably with sea salts.

19. A method according to any one of claims 1 to 18, wherein said culture is performed as a batch process or a fed-batch process.

20. A method according to claim 19, wherein the culture is performed for between about 4 to about 10 days, preferably between about 6 to about 9 days.

21. A method according to any one of claims 1 to 18, wherein said culture is performed as a continuous process or semi-continuous process.

22. A method according to any one of claims 1 to 21, wherein the method further comprises extracting oil including docosahexaenoic acid from the microorganism and, preferably, purifying the oil to increase the docosahexaenoic acid content of the oil.

23. A method according to any one of claims 1 to 22, wherein the method further comprises the purification or partial purification of docosahexaenoic acid from the microorganism.

24. A method according to any one of claims 1 to 23, wherein the culture does not include a stationary phase.

25. An oil comprising docosahexaenoic acid prepared from a microorganism cultured in accordance with any one of claims 1 to 24.

26. An at least partially purified preparation of docosahexaenoic acid prepared from a microorganism cultured in accordance with any one of claims 1 to 24.

27. A method according to any one of claims 1 to 24, wherein the initial concentration of the compound is about 8 g/l.

28. A method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionised form of an acidic group, the microorganism synthesising docosahexaenoic acid containing carbon from the species.

29. A method of culturing a microorganism for the synthesis of a polyunsaturated fatty acid by the microorganism, comprising culturing *C. cohnii* with an organic species comprising an acidic group or an ionised form of an acidic group, the *C. cohnii* using the species as a carbon source and synthesising a polyunsaturated fatty acid.

30. An oil comprising said polyunsaturated fatty acid of claim 29, prepared from a microorganism cultured in accordance with claim 29.

31. An at least partially purified preparation of said polyunsaturated fatty acid of claim 29, prepared from a microorganism cultured in accordance with claim 29.

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32. A microorganism cultured in accordance with any one of claims 1 to 24, 28 or 29.

33. A method comprising using a microorganism according to claim 32 as a food or a food supplement.

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